The Electrochemistry of Proteins entrapped in Nafion

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The electrochemistry of cytochrome c_{551}, cytochrome b_5, and azurin at a Nafion-coated electrode is described.

Following the original observation[1,2] of the direct electrochemistry of cytochrome c, much work has been reported[3] on the conditions which allow rapid electron transfer to proteins at an electrode surface; in particular the concept of surface modification and/or changes in the solution conditions by which proteins adsorb reversibly in a manner appropriate for electron transfer has been explored. We were interested to see if it would be possible to 'entrap' proteins at an electrode surface in such a manner that they were able to dispose themselves so that the prosthetic group was close to the electrode surface.

We report herein the incorporation of proteins into the polyelectrolyte Nafion (E. I. du Pont de Nemours) by direct mixing in a ratio of 1 : 3 of, *e.g.*, cytochrome c_{551} (1.3 mM) and a 5% solution of the polymer, dissolved in sodium phosphate (0.1 M; pH 7.2), to give a final polymer concentration of 1.66%. This solution was then spread on to the cleaned surface of a basal plane graphite electrode (surface area 0.13 cm^2) and air-dried. To ensure stability of the electrode, a second layer *of the polymer alone*, was added. Routinely obtained cyclic voltammograms of bacterial cytochrome c_{551} at high ionic strength, give rise to the data in Table 1. The electrochemistry of this protein is quasi-reversible and the

Table 1. Properties and apparent redox potentials of Nafion immobilized proteins.

Protein	Charge	*M*/kDa	E'_0 [a]/mV	$E_{1/2}$ [b]/mV
Cyt c_{551}	−1/−2	10.0	+18	+60
Cyt b_5	−7/−8	15.6	−237	−300
Azurin	+1/+2	14.0	+88	+225
Cyt c	+7/+8	12.4	+28	—

[a] Formal potential with respect to a saturated calomel electrode. [b] Half-wave potential.

apparent $E_{1/2}$ (+60 mV) is somewhat dependent on the ionic strength. Of the proteins investigated, *all*, with the exception of cytochrome c, display (see Table 1) appropriate responses. *No electrochemistry is observed for cytochrome c.*

We interpret these data as being associated with the heterogenous nature of the polymer matrix.[4] All the proteins are entrapped by the polymer but it is only in the case of negatively charged, or weakly positively charged proteins, that they interact with the electrode surface. The nature of the interaction is currently being investigated but it appears that it depends only on the state of the proteins at the electrode/polymer interface and does not depend on, for example, diffusion of the proteins through the polymer. The highly positively charged cytochrome c binds too tightly to the polymer and hence is unable to interact with the electrode surface.

Preliminary results[5] suggest that not only anionic proteins may be immobilised in this way; the method may also be extended to the entrapment of redox enzymes with retention of their activity.

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References

1 M. J. Eddowes and H. A. O. Hill, *J. Chem. Soc., Chem. Commun.*, 1977, 771.
2 Y. Yeh and T. Kuwana, *Chem. Lett.*, 1977, 1145.
3 F. A. Armstrong, H. A. O. Hill, and N. J. Walton, *Acc. Chem. Res.*, 1988, **21**, 407.
4 H. L. Yeager and A. Steck, *J. Electrochem. Soc.*, 1981, **128**, 1880.
5 C. E. W. Hahn, H. A. O. Hill, M. D. Ritchie, and J. W. Sear, to be published.